HAYATI
Journal of Biosciences

Vol. 32 No. 1, January 2025 233-240
DOI:10.4308/hjb.32.1.233-240
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Check for updates

OPEN ACCESS

The Survival, Growth, and Accelerating Morphological Development of *Stichopus horrens* are Affected by the Initial Larval Stocking Densities

Sari Budi Moria Sembiring[1*], Ketut Maha Setiawati[1], Zeny Widiastuti[2], Jhon Harianto Hutapea[1], Gunawan Gunawan[1], Ananto Setiadi[1], Haryanti[1], Nyoman Adi Asmara Giri[1]

[1]*Research Center for Fishery, National Research and Innovation Agency (BRIN), Cibinong, Bogor, Indonesia*
[2]*Directorate General of Aquaculture, Brackishwater Aquaculture Development Center, Situbondo 68351, Indonesia*

ARTICLE INFO

Article history:
Received May 26, 2024
Received in revised form September 4, 2024
Accepted October 31, 2024

KEYWORDS:
Density,
Growth,
Morphological development,
Stichopus horrens,
Survival rate

ABSTRACT

Stichopus horrens is highly exploited due to their use as a pharmaceutical ingredient. Since then, this species has become extinct; therefore, it is necessary to start aquaculture. Gonad maturation and broodstock spawning succeeded, but the optimum larval-rearing stocking density has not yet been determined. Therefore, the present study aimed to determine the optimal stocking density to improve survival and accelerate the development of larval morphology. Three treatments were used: 100, 150, and 200 larvae L^{-1}, each with four replicates. Twelve 100-litre plastic containers filled with 80 litres of seawater as larval rearing media were placed in a concrete tank with a water-bath system using a heater and a thermostat (29.0°C±1.0°C). The larvae were fed a mixture of microalgae (*Chaetoceros muelleri, Isochrysis galbana*, and *Nitzchia* sp.) twice a day^{-1}. The data collected included survival and growth rates, larval morphological development, and water quality. The fastest metamorphose from auricularia to the doliolaria stage occurred in 100 larvae L^{-1}, 15 days after hatching. The highest survival rate, growth rate, and percentage of larvae metamorphose to the doliolaria stage were obtained in the 100 larvae L^{-1} as 26.38%, 26.4 µm day^{-1}, and 65.27%, respectively, and were significantly different ($P<0.05$). A stocking density of 100 larvae L^{-1} was optimal for promoting survival and growth and accelerating the morphological development of auricularia to the doliolaria stage.

1. Introduction

Sea cucumbers, classified as echinoderms, are typically economically valuable, particularly *Stichopus* species (Purcell *et al.* 2023). Traditional fishermen call the stichopus group a "gamete" (Sulardiono *et al.* 2022). Sea cucumbers are rich in protein, vitamins, minerals, and fatty acids (Rasyid *et al.* 2020; Mazlan *et al.* 2023). It contains important bioactive compounds and contributes to health benefits, including anti-inflammatory, antioxidant, antimicrobial, antitumor, antihypertensive, anticoagulant, anti-angiogenic, antithrombotic, wound healing, and anticancer effects (Lu *et al.* 2022). Owing to excessive exploitation, the presence of these species has become scarce and elusive. Therefore, it is imperative to establish a sea cucumber aquaculture system. The primary obstacle to advancing sea cucumber farming is the limited availability of seed sea cucumbers; therefore, it is necessary to generate seeds from hatcheries.

Reproductive studies on sea cucumbers in Indonesia have begun with *Holothuria scabra*. Spontaneous spawning in captivity, larval culture, and juvenile rearing have been successful (Sembiring *et al.* 2015; Indriana 2019). The sea cucumber *Stichopus horrens* hatchery was initiated in 2019 by collecting and rearing candidate broodstocks in captivity, feeding them with seaweed (Widiastuti *et al.* 2019),

* Corresponding Author
E-mail Address: moriasembiring@gmail.com

and spawning began in 2020. Larval rearing started and succeeded even though the survival rate to the juvenile stage is still extremely low (0.05-0.13%). Many variables contribute to juvenile sea cucumber production, including the quality of the broodstocks and fertilized eggs, water quality, food management, space availability, and hatchery procedures such as larval rearing (Laguerre *et al.* 2020).

The mortality rate of sea cucumber larvae during the rearing process can be due to the initial density of larvae, which is an external factor that affects the survival, growth, and metamorphosis of the larvae (Acosta *et al.* 2020). The success of holothuria larval culture is influenced by the density at which the larvae are reared, and the optimal density varies depending on the species. An ideal density for rearing *Holothuria scabra* larvae is considered to be between 0.5-1.5 larvae ml^{-1}, as suggested by Asha and Diwakar (2013) and Abidin *et al.* (2019). On the other hand, a density of 0.2 larvae ml^{-1} is recommended for Apostichopus japonicus larvae, according to Ru *et al.* (2022), while *Holothuria nobilis* larvae are best reared at a density of 0.6 larvae ml^{-1}, as stated by Nguyen *et al.* (2021).

The density of larvae is correlated with feed consumption and nutrient adequacy as the unhindered movement of the ciliary band with which the larvae swim and ingest food. Furthermore, morphological development is also affected by nutrient adequacy. The optimum stocking density for rearing *Stichopus horrens* larvae is still unknown, so conducting experiments on different initial stocking densities is necessary. By determining the optimal density that increases the survival and acceleration of morphological development of larvae, this experiment will contribute to the development of culture technology, as this species has high commercial value in Indonesia.

2. Materials and Methods

The current study was conducted in the Scientific Conservation Region for Marine Organisms, Research Center for Fishery, National Research and Innovation Agency, Gondol-Bali, Indonesia.

2.1. Collection of Animals and Spawning

Twenty wild-caught *Stichopus horrens* broodstocks were directly reared in a 4,000-liter tank of fiber-reinforced plastic. The tank was then filled with sand-filtered seawater. Mean water temperature and salinity were 29±1°C and 33±1 ppt, respectively, and the daily water exchange rate was 50%. The broodstock was fed with *Sargassum* sp. and *Ulva* sp. (2:1) at 3% of their body weight daily (Widiastuti *et al.* 2019). The average weight of the broodstock used in this experiment was 280.1±23.0 g (± SD; wet weight), and the mean total length was 20.1±4.4 cm (Setiawati *et al.* 2021).

As a spawning tank, the broodstock was transferred to a 200-litre transparent fibreglass tank during the new moon phase as a natural spawning period. The tank was filled with filtered (a 1 µm cartridge filter) and UV radiation-sterilized seawater. The spawning tank was covered with a black plastic sheet to allow the water temperature to increase by 1-2°C above the ambient temperature (29°C) to induce the broodstock to spawn. After spawning, the broodstock was removed and transferred to the broodstock tank. Water from the spawning tank was siphoned and drained into an 80 µm plankton net using a water hose to collect the eggs. Then, the eggs were washed with filtered and UV-sterilized seawater to remove excess sperm and transferred into a 200-litre incubation tank. Twenty-four hours after spawning, the eggs hatched into larvae, and shiponing was performed to remove the unhatched eggs. Then, the larvae with more uniform numbers were ready to be transferred into the different larval density experiments.

2.2. Larval Rearing Experiment

The determination of larval stocking density treatment pertains to studies conducted on various species of sea cucumbers (Asha and Diwakar 2013; Acosta *et al.* 2020). The stocking densities for the larvae 1 d after hatching (DAH) rearing tests were 100, 150, and 200 larvae L^{-1}. The morphological development observed during the experiment was *auricularia* stages (early, mid, and late *auricularia*), followed by metamorphosis into the *doliolaria* stage at approximately 21 DAH (Ren *et al.* 2017). Larvae at *auricularia* are transparent and planktonic with slow movement. The digestive system begins to form in early *auricularia*, vibrating bristles begin to form in mid-*auricularia*, and in late *auricularia*, four lateral protrusions begin to form, and at each edge, a hyaline sphere is present. The *Doliolaria* stage is a transition from planktonic to settlement larvae, with rapid movement. The body shape changed from flat to tube-like and brown in color, with five hyaline spheres on each side (Figure 1).

The experiment used 12 plastic containers, each with a capacity of 100 litres (20 cm in diameter and 16 cm in height). Each treatment consisted of four replicates.

All experimental tanks were placed in a 270 × 175 × 70 cm concrete tank that functioned as a water bath system. During the experiment, the water temperature was controlled at 29.0±1.0°C using a heater and an automatic thermostat type 611-H regulate. Soft aeration (59.0±0.5 sec L^{-1}) was applied to supply oxygen and keep larvae distributed in the water column. To maintain good water quality, every two days, water was drained from the larval rearing tank at 10% during the early auricularia stage and increased to 20% as the larvae developed to mid-auricularia and further stage.

The larvae were fed two feedings per day consisting of a mixture of microalgae (*Chaetoceros muelleri, Isochrysis galbana*, and *Nitzchia* sp.) in certain proportions based on their stage of development (Table 1) (Sembiring *et al.* 2015; Matrosova *et al.* 2020; Yu *et al.* 2022). The diatom group (*Chaetoceros muelleri, Nitzchia* sp.) was cultivated in Na medium, whereas the non-diatom group (*Isochrysis galbana*) was cultured in MQ (Miquel) medium (Perumal *et al.* 2015).

To assess the larval survival rate based on the number of larvae in one period divided by the initial number

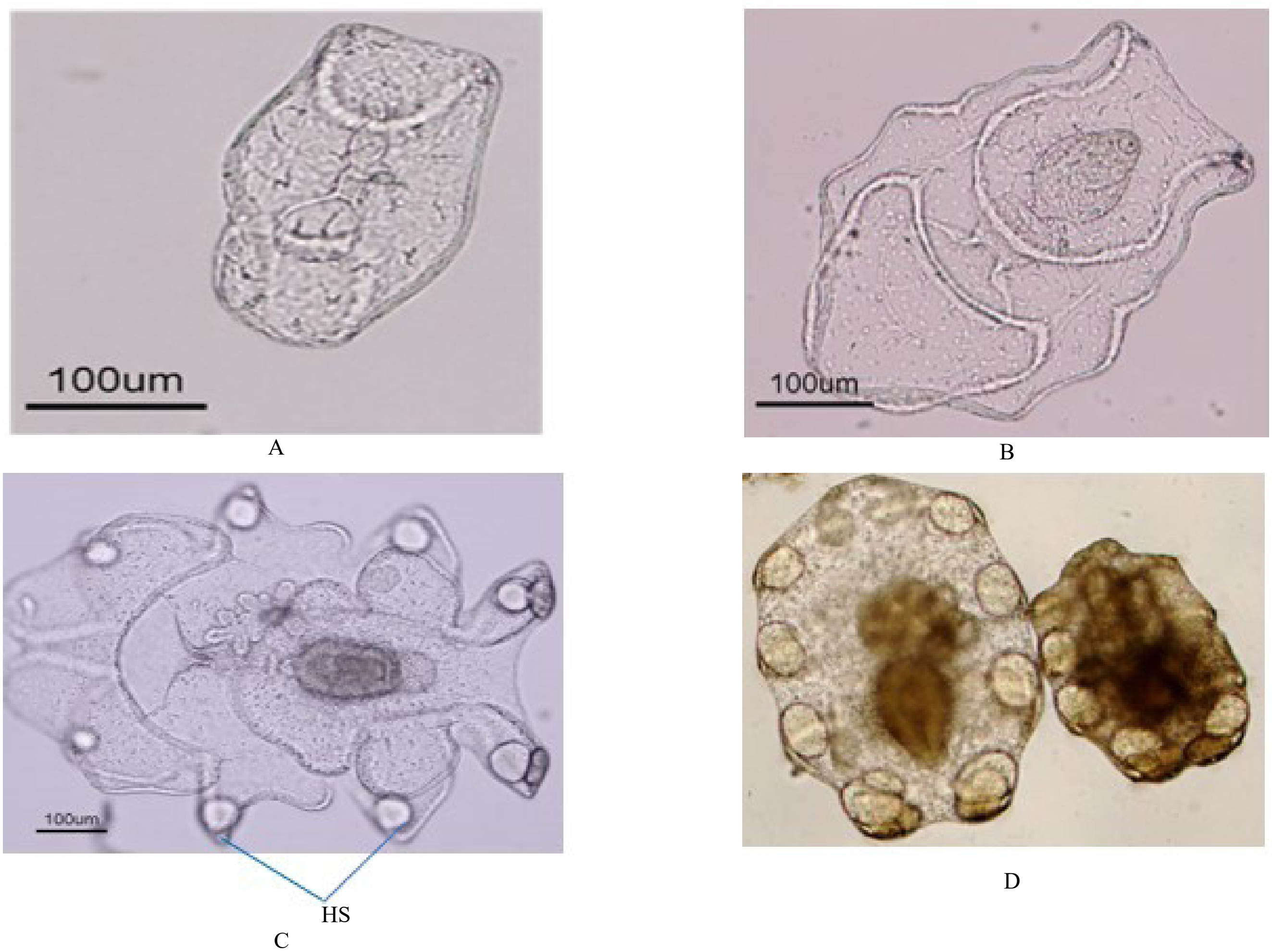

Figure 1. *Stichopus horrens* larval development of (A) early *auricularia* (1 DAH); (B) mid- *auricularia* (8 DAH); (C) late *auricularia* with hyaline spheres (15 DAH); hyaline spheres (HS); and (D) *doliolaria* (21 DAH)

Table 1. Feeding regimes for *Stichopus horrens* larvae

Microalgae	Food composition (× 10^3 cells ml^{-1})			
	Early *auricularia*	Mid-*auricularia*	Late *auricularia*	*Doliolaria*
Chaetoceros muelleri	5-15	20	5-15	20
Isochrysis galbana	0	10-20	0	10-20
Nitzchia sp.	0	0	0	0

multiplied by 100%, morphological development, length, and width, three random subsamples of 100 ml were collected from each treatment and replicated at 1, 2, 6, 7, 8, 14, 17, and 21 after hatching (DAH). Samples collected at 1 and 2 DAH as critical initial feeding, 6-8 DAH is the estimated time for development to mid-auricularia, and so on. The growth and morphological development of ten larvae were measured (Figure 2) and observed (Figure 1) using a binocular microscope attached to a Nikon DXM 1200F camera and Win-Roof version 5.0. Physicochemical parameters, such as temperature, salinity, pH, dissolved oxygen, nitrite, and ammonia, were measured every five days.

2.3. Statistical Analysis

The analysis was conducted using SPSS statistical program version 2.0. Before analysis, normally assumptions were evaluated using the Kolmogorov-Smirnov test, and homogeneity of variance was examined using the Levene test. The treatment parameters were analyzed using a one-way ANOVA to determine their differences. A post hoc multiple comparison test (Tukey's HSD) revealed significant variations among the means. Results were deemed statistically significant if the probability value was $P<0.05$.

3. Results

3.1. Survival Rate, Growth Rate, and Morphology Development of *Stichophus horrens* Larvae

The survival rate of larvae in the treatment 100 larvae L^{-1} (86.48±1.99%) is greater until 11 days of culture compared to the treatments 150 and 200 larvae L^{-1} (79.49±3.96% and 76.67±2.86% [±SE]). Despite a decrease in the survival rate starting from day 14, the ultimate survival rate on day 21 remained higher in the treatment with 100 larvae L^{-1} (26.38±1.86%). It was substantially different ($P<0.05$) compared to the treatments with 150 and 200 larvae L^{-1} (18.84±0.89% and 14.53±0.97%, respectively) (Figure 3). Post hoc testing indicated that a larval density of 100 L^{-1} resulted in the highest survival rate.

All larvae at the beginning of the experiment (1 DAH) had the same length, but between 6 and 8 DAH, and there was a noticeable variation among the different treatments. Microscopic observations showed that the larvae began to feed at 2 DAH, marked by the brown color of the larval intestine (Figure 4).

The treatment with 100 larvae L^{-1} had the longest length, as shown in Table 2. From day 14 until the end of the trial, there was a significant difference in

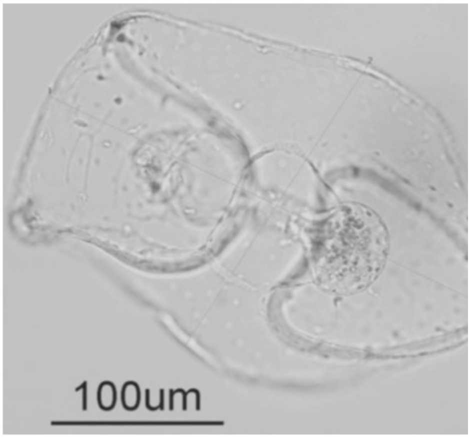

Figure 2. Size parameters of the *auricularia* larvae. W (width) and L (length)

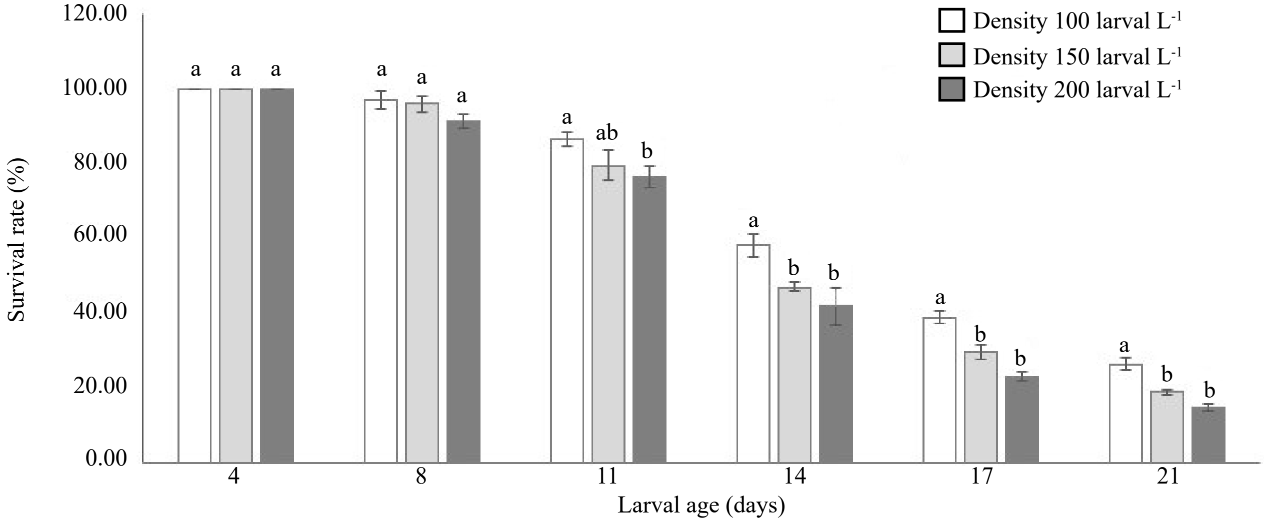

Figure 3. Survival rate of *Stichopus horrens* larvae at different culture densities. Bars indicate standard errors. Letters denote significant differences among the treatments

length between the treatments with 100 larvae L^{-1} and 200 larvae L^{-1} ($P<0.05$), but not significantly different with 150 larvae L^{-1} ($P>0.05$). The width of the larvae exhibited a statistically significant difference ($P<0.05$) between the 100 larvae L^{-1} and 200 larvae L^{-1} treatments during the experiment, but there was no significant difference between treatments with 100 larvae L^{-1} and 150 larvae L^{-1} ($P>0.05$) from day 14 to the completion of the experiment.

After the experiment ended, the larvae in the treatment with a density of 100 larvae L^{-1} showed a growth rate of 26.4 µm per day and an average length of 900.00±46.84 µm (± SE). This growth rate was significantly higher ($P<0.05$) compared to the densities of 150 and 200 larvae L^{-1}, with growth rates of 13.5 µm day^{-1} and 10.5 µm day^{-1}, and an average length of 859.44±38.62 µm and 800.00±48.33 µm, respectively (Table 2).

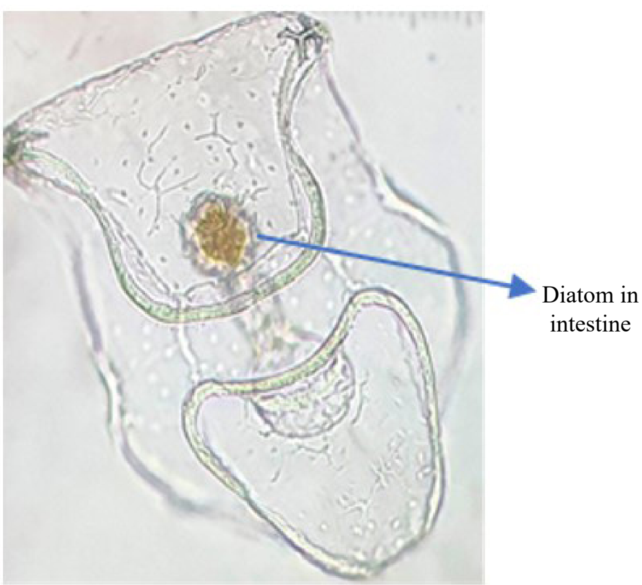

Figure 4. The initial feeding of *Stichopus horrens* larvae on diatoms occurred at 2 DAH

The three stages of auricularia were observed until 17 DAH, and all larvae were still in the early auricularia stage until 6 DAH, with a gradual increase in length. At 8 DAH, the larvae reached the mid-auricularia stage at 6.46%, 3.39%, and 1.43% at 100, 150, and 200 larvae L^{-1}, respectively. The morphological development of the auricularia larvae stage in the 100 larvae L^{-1} treatment was significantly higher than that in the 200 larvae L^{-1} treatment ($P<0.05$), but not significantly different from the 150 larvae L^{-1} treatment. Further development into late auricularia started at 14 DAH, as shown by the formation of hyaline spheres. At the densities of 100 larvae, hyaline spheres were observed for the first time on day 11, and the hyaline spheres were as high as 6.45%. In the 150 and 200 larvae L^{-1} treatments, hyaline spheres were only detected from 17 DAH onwards at percentages of 7.63% and 3.97%, respectively. At the end of the experiment (21 DAH), the rate of the doliolaria stage at a density of 100 larvae L^{-1} (65.27%) was the highest and significantly different ($P<0.05$) from the other treatments. The morphological development of larvae reared at densities of 150 and 200 larvae L^{-1} was 19.27% and 10.23%, respectively, and was not significantly different ($P>0.05$) (Figure 5).

3.2. Water Quality

Water quality parameters such as temperature, salinity, pH, dissolved oxygen, NO_2, and NH_3 during larval rearing are still within a reasonable range to support the morphological development and growth of *Sticophus horrens* larvae (Table 3).

4. Discussion

The survival and growth of *Stichopus horrens* larvae were significantly affected by stocking density. The highest length gain at the stocking density of 100 larvae L^{-1} could be attributed to low competition for

Table 2. Length and width (± SE) of *Stichopus horrens* larvae under three different larval densities with four replicates (n = 10 larvae, early, mid, and late auricularia continuously increased while *doliolaria* stage shortened)

Larval stage	Day (D)	Length (µm)			Width (µm)		
		100 larval L^{-1}	150 larval L^{-1}	200 larval L^{-1}	100 larval L^{-1}	150 larval L^{-1}	200 larval L^{-1}
Early *auricularia*							
Start	1	397.81±25.24^{a}	397.81±25.24^{a}	397.81±25.24^{a}	287.84±14.28^{a}	290.75±17.95^{ab}	280.50±11.10^{b}
End	6	650.00±13.80^{a}	530.00±9.51^{b}	500.00±46.11	328.00±13.41^{a}	327.00±3.02^{ab}	319.50±46.49^{b}
Mid *auricularia*							
Start	7	750.00±18.50^{a}	566.25±20.81^{b}	515.63±31.25^{b}	469.07±2.04^{a}	444.19±10.09^{ab}	415.31±32.78^{b}
End	8	810.00±14.80^{a}	664.75±38.60^{b}	640.75±36.32^{b}	509.34±3.30^{a}	485.03±15.39^{ab}	427.88±16.35^{b}
Late *auricularia*							
Start	14	1,116.26±13.45^{a}	1,020.00±20.23^{a}	813.84±25.05^{b}	965.00±1.76^{a}	736.20±18.83^{b}	646.62±16.40^{b}
End	17	959.25±12.06^{a}	833.73±26.85^{ab}	864.63±19.19^{b}	709.06±0.89^{a}	887.19±14.17^{b}	731.74±28.28^{b}
Doliolaria	21	900.00±46.84^{a}	859.44±38.62^{ab}	800.00±48.33^{b}	595.57±6.42^{a}	954.66±32.99^{b}	878.64±33.26^{b}

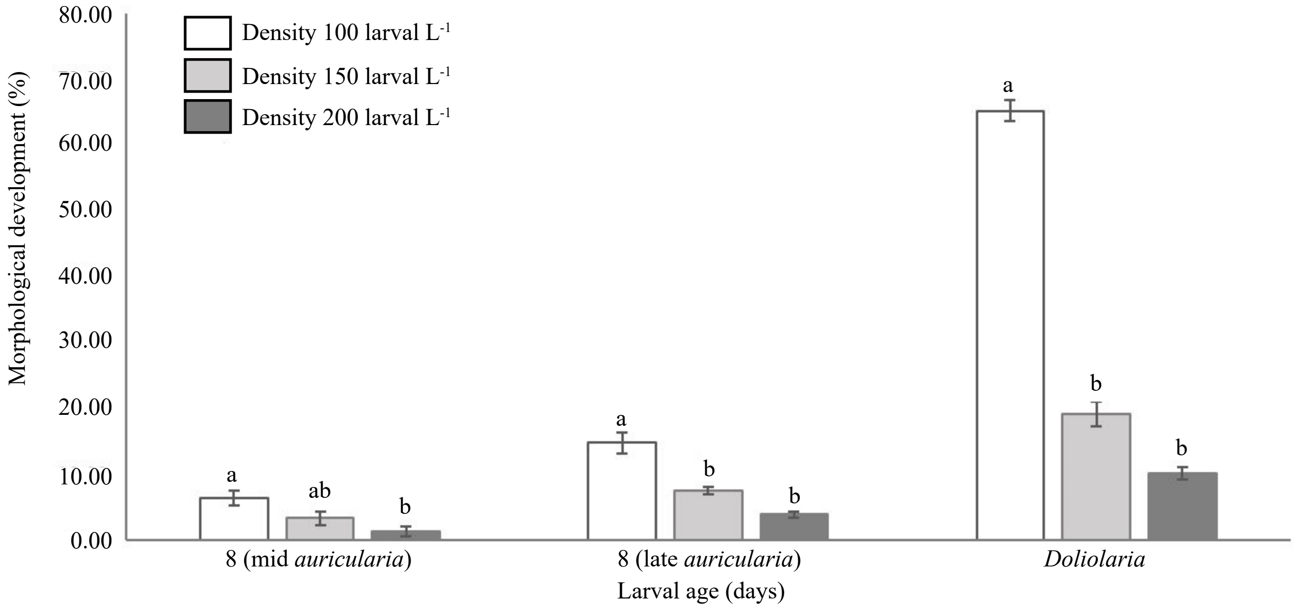

Figure 5. Morphological development of *Stichopus horrens* larvae at three different larval densities. Bars indicate standard errors. Letters denote significant differences among treatments

Table 3. Values of water quality parameters (± SD) measured during the rearing of *Stichopus horrens* under three different larval densities with four replicates

Density treatment (larval L^{-1})	Temperature (°C)	Salinity (ppt)	pH	Dissolved oxygen (mg L^{-1})	NO_2 (mg L^{-1})	NH_3 (mg L^{-1})
100	29.27±0.17	34.0±1.0	8.14±0.07	5.17±0.08	0.11±0.03	0.37±0.02
150	29.28±0.13	34.0±1.0	8.15±0.08	5.18±0.08	0.15±0.07	0.38±0.03
200	29.27±0.19	34.0±1.0	8.13±0.06	5.17±0.03	0.17±0.05	0.38±0.05

feed compared to the stocking density of 200 larvae L^{-1}. Feed is an external factor with the most prominent effect on the growth of organisms. The high density of larvae causes an abrupt cessation of the movement of the ciliary band, with which the larvae swim and ingest food, resulting in reduced feed consumption and nutrient depletion (Ren *et al.* 2017). A high stocking density leads to feed scarcity, resulting in limited access to sufficient food for larvae, and consequently delayed growth and significant variations in size (Sun and Li 2012; Acosta *et al.* 2020). According to Sewell *et al.* (2023), when sea cucumber larvae are crowded together in high numbers, there is less space and food availability. This lack of resources leads to malnutrition, hindered development and growth, and decreased chance of survival.

The feed composition plays an important role in the formation of hyaline spheres during the late *auricularia* stage. The formation of hyaline spheres indicates that the larvae were able to digest the microalgae perfectly (Ren *et al.* 2015). The decrease in survival rates at 15 DAH was ascribed to morphological alterations during the late auricularia stage, ultimately resulting in the doliolaria stage (metamorphosis process). Hyaline spheres are energy reserves that play a principal role in metamorphosis from late *auricularia* to *doliolaria*. According to Ren *et al.* (2017), during metamorphosis from late *auricularia* into *doliolaria*, larvae do not ingest feed but use nutrient reserves in the hyaline spheres. In the *doliolaria* stage, hyaline spheres switch their function to locomotion and further transform into tube feet ambulacral in the pentactula stage (Peters-Didier and Sewell 2017). The larvae exhibited the lowest survival rate when the stocking density was set at 150 and 200 larvae L^{-1}. This phenomenon can be ascribed to intense competition for resources such as habitat and nourishment. In *Holothuria scabra*, a comparable occurrence was noted, with the lowest survival rate of larvae observed when the stocking density exceeded 200 larvae L^{-1} (Serang *et al.* 2016).

The growth performance of *Stichopus horrens* larvae with different stocking densities was higher than that of other reported sea cucumber species. For example, the maximum size of larvae *Stichopus horrens* at the late *auricularia* stage was (1,116.26±13.45 µm), which is higher than *Holothuria mammata* that attained late auricularia stage with a mean length of 433 µm (Domínguez-Godino and González-Wangüemert 2018), *Holothuria polii* mean length of 705 µm (Arnold *et al.* 2018), and *Holothuria nobilis* mean length 961.3 ± 8.19 µm (Nguyen *et al.* 2021). Asha and Diwakar (2013) noted that the size of *auricularia* larvae differs among species, which influences the ideal stocking density for larval rearing. When many larvae are in a confined space, there is a higher chance of death (Tuwo and Tresnati 2015).

In conclusion, the initial stocking density significantly influenced the growth, survival, and morphological development of *Stichopus horrens* larvae. The stocking density of 100 larvae L^{-1} was optimum for supporting their growth and survival rate, and could accelerate their metamorphoses to the *doliolaria* stage.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors are grateful to the Scientific Conservation Region for Marine Organisms, Gondol-Bali, Research Center for Fishery, National Research and Innovation Agency, and Sudira Hendrawan, CV. Inti Resource Aquatic, for supporting the research funding. The authors also recognized valuable technical assistance from I Made Buda, Siyam Sujarwani, I Nengah Gde Suparta, Dadang Rusmana, Ahmad Rifa'i, I Nengah Suriadnyani, and Luh Yuliani Dewi.

References

Abidin, N.A., Muhamad Saleh, S.R., Ching, F.F., Othman, R., Manjaji-Matsumoto, M., Mustafa, S., Senoo, S., 2019. Appropriate diet and stocking density for sea cucumber (*Holothuria scabra*) larvae rearing. *J. Phys.: Conf. Ser.* 1358, 1-9. https://doi.org/10.1088/1742-6596/1358/1/012015

Acosta, E.J., Rodriguez-Forero, A., Werding, B., Kunzmann, A., 2020. Effect of density, temperature, and diet on the growth, survival, and development of larvae and juveniles of *Isosticophus* sp. *Aquaculture Research*. 52, 611-624. https://doi.org/10.1111/are.14918

Arnold, R., Fianchini, A., Boncagni, P., Lovatelli, A., Scardi, M., Cataudella, S., 2018. Spawning and rearing of *Holothuria tubulosa*: a new candidate for aquaculture in the Mediterranean region. *Aquaculture Research*. 49, 557–568. https://doi.org/10.1111/are.13487

Asha, P.S., Diwakar, K., 2013. Effect of stocking density on the hatching rate, larval and early juvenile rearing of edible sea cucumber *Holothuria scabra* (Jaeger, 1883). *Indian Journal of Geo-Marine Sciences*. 42, 191–195.

Domínguez-Godino, J.A., Mercedes González-Wangüemert, M., 2018. Breeding and larval development of *Holothuria mammata*, a new target species for aquaculture. *Aquaculture Research*. 49, 1430–1440. https://doi.org/10.1111/are.13597

Indriana, L.F., 2019. Spawning, incubation of eggs, and larval rearing techniques of sandfish *Holothuria scabra*. In Biology and cultivation of sandfish, *Holothuria scabra* Book. AMAFRAD Press. 27-37.

Laguerre, H., Raymond, G., Plan, P., Ameziane, N., Bailly, X., Le Chevalier, P., 2020. First description of embryonic and larval development, juvenile growth of the black-sea cucumber Holothuria forskali (Echinodermata:Holothuroidea), a new species for aquaculture in the north-eastern Atlantic. *Aquaculture*. 521, 734961. https://doi.org/10.1016/j.aquaculture.2020.734961

Lu, Z., Sun, N., Dong, L., Gao, Y., Lin, S., 2022. Production of bioactive peptides from sea cucumber and its potential health benefits: a comprehensive review. *Journal of Agricultural and Food Chemistry*. 70, 7607–7625. https://doi.org/10.1021/acs.jafc.2c02696

Matrosova, I.V., Politaeva, A.A., Ilyuschenko, V.V., Panchishina, E.M., Shadrina, E.V., Kornienko, N.L., 2020. Experimental larval rearing of the Japanese sea cucumber (*Apostichopus japonicus*) in Severnaya Bay (Slavyansky Bay, sea of Japan). I*OP Conf. Ser.: Earth Environ. Sci.* 548, 1-6. https://doi.org/10.1088/1755-1315/548/7/072059

Mazlan, N.B., Rahman, N.N.A., Shukhairi, S.S., Nazahuddin, M.N.A.B., 2023. Sea cucumbers: source of nutritional, medicinal, and cosmeceutical products, in: Shah, M.D., Ransangan, J., Venmathi Maran, B.A. (Eds.), *Marine Biotechnology: Applications in Food, Drugs and Energy*. Springer, Singapore, pp. 171-188.

Nguyen, H.V., Mariamu, B.A., Pham, H.Q., 2021. Induced spawning and larval rearing of the sea cucumber *Holothuria nobilis. The Israeli Journal of Aquaculture*. 73, 1-16. https://doi.org/10.46989/001c.28235

Perumal, P., Balaji Prasath, B., Perumal, S., Ananth, S., Shenbaga Devi, A., Dinesh Kumar, S., Jeyanthi, S., 2015. Isolation and culture of microalgae, in: Perumal, S., A.R., T., Pachiappan, P. (Eds.), *Advances in Marine and Brackishwater Aquaculture*. Springer, New Delhi, pp. 1-15. https://doi.org/10.1007/978-81-322-2271-2_1

Peters-Didier, J., Sewell, M.A., 2017. Maternal investment and nutrient utilization during early larval development of the sea cucumber *Australostichopus mollis. Marine Biology*. 164, 178. https://doi.org/10.1007/s00227-017-3209-7

Purcell, S.W., Lovatelli, A., González-Wangüemert, M., Solís-Marín, F.A., Samyn, Y., Conand, C., 2023. Commercially Important Sea Cucumbers of the World, second ed. FAO, Rome.

Rasyid, A., Murniasih, T., Putra, M.Y., Pangestu, R., Harahap, I.A., Untari, F., Sembiring, S.B.M., 2020. Evaluation of nutritional value of sea cucumber *Holothuria scabra* cultured in Bali, Indonesia. *AACL Bioflux.* 13, 2083-2093.

Ren, Y., Liu, W., Dong, S., Pearce, C.M., 2015. Effects of mono-species and bi-species microalgal diets on the growth, survival, and metamorphosis of auricularia larvae of the California sea cucumber *Parastichopus californicus* (Stimpson, 1857). *Aquaculture Nutrition.* 22, 304-314. https://doi.org/10.1111/anu.12253

Ren, Y., Liu, W., Pearce, C.M., 2017. Effects of stocking density, ratio and temperature on growth, survival, and metamorphosis of auricularia larvae of the California sea cucumber, *Parastichopus californicus. Aquaculture Research.* 49, 517–525. https://doi.org/ARE.13482

Ru, X., Feng, Q., Zhang, S., Liu, S., Zhang, L., Yang, H., 2022. Eco-friendly method for rearing sea cucumber (*Apostichopus japonicus*) larvae. *Aquaculture Research.* 53, 3759 - 3766. https://doi.org/10.1111/are.15880

Sembiring, S.B.M., Hutapea, J.H., Sugama, K., Susanto, B., Giri, N.A., Haryanti, 2015. Breeding technology of sandfish (*Holothuria scabra*), in: Soekadi, F., Sugama, K., Nurhakim, S., Heruwati, E.S., Purba, M., Kusnendar, E., Djunaidah, I.S., Sudibjo, E.R., Sakti, I. (Eds.), *Technology Recommendation Marine and Fisheries*. Agency for Research and Development Marine and Fisheries, Ministry of Marine and Fisheries Affairs, Jakarta, pp.187-200.

Serang, A.M., Santi, P.T.R., Pitjont, T., 2016. Effect of different stocking densities on growth and survival rates of Sea cucumber *Holothuria scabra* seedlings. *Aquaculture Indonesiana*. 17, 30-34. https://doi.org/10.21534/ai.v17i1.44

Setiawati, K.M., Widiastuti, Z., Sembiring, S.B.M., Giri, N.A., 2021. Growth and reproduction performance of sea cucumber (*Stichopus* sp.) fed with different feed regime. *E3S WEB of Conferences.* 322, 1-10. https://doi.org/10.1051/e3sconf/202132202009

Sewell, M.A., Hamel, J.F., Mercier, A., 2023. Chapter 16: Morphological diversity, development, and biology of sea cucumber larvae, in: Mercier, A., Hamel, J.F., Pearce, C.M., (Eds.), *The World of Sea Cucumbers.* Academic Press, pp. 237-256. https://doi.org/10.1016/B978-0-323-95377-1.00018-7

Sulardiono, B., Hartoko, A., Aini, A. N., Wulandari, D., Budiharjo, A., 2022. Genetic diversity of commercial sea cucumbers Stichopus (Echinoderm: Stichopodidae) based on DNA Barcoding in Karimunjawa, Indonesia. *Biodiversitas*. 23, 922–927. https://doi.org/ 10.13057/biodiv/d230234

Sun, X., Qi Li., 2012. Effects of temporary starvation on larval growth, survival and development of the Sea cucumber *Apostichopus japonicus. Marine Biology Research.* 8, 771–777. https://doi.org/10.1080/17451000.2012.676186

Tuwo, A., Tresnati, J., 2015. Echinoderm aquaculture sea cucumber farming in Southeast Asia (Malaysia, Philippines, Indonesia, Vietnam), in: Brown, N.P., Eddy, S.D. (Eds.), *Echinoderm Aquaculture.* Wiley-Blackwell,. pp. 331-352. https://doi.org/10.1002/9781119005810.ch15.

Widiastuti, Z., Setiawati, K.M., Sembiring, S.B.M., Giri, N.A. 2019. Utilization of seaweed to support sea cucumber domestication activities (*Stichopus* sp.). *In: Proceedings of the XVI Annual National Seminar on Fisheries and Marine Research*. Yogyakarta: Gadjah Mada University. pp. 50-54.

Yu, Z., Wu, H., Tu, Y., Hong, Z., Luo, J., 2022. Effects of diet on larval survival, growth, and development of the sea cucumber *Holothuria leucospilota. Aquaculture Nutrition.* 2022, 1-10. https://doi.org/10.1155/2022/894799710